nucleic acid sequence according to Claim 31;

(b) maintaining said host cell under conditions allowing the expression of glial cell line-derived neurotrophic factor by said host cell; and

(c) optionally, isolating the glial cell line-derived neurotrophic factor expressed by said host cell.

Please cancel claims 1-25, 37-41 and 56-74 (as identified in the May 5, 1995 restriction requirement) without prejudice as to the subject matter contained therein. These claims and others will be pursued in the divisional and continuation applications. In addition, please cancel claims 35 and 36, the content of which has been inserted into the amended claims, above. Claims 45, 47, 48, 51, 53 and 54 have not been amended, but are included above for ease of reference in view of the extensive claim revisions.

## REMARKS

Reference to the parent application has been revised as suggested by the Examiner. Support for amendments cross-referencing the Figures and the Sequence Listing can be found in the original Sequence Listing and Figures 8, 9, 12-14, 19 and 22. Support for the revised Sequence Listing suggested by the Examiner (pages 92-99D) can be found in the original Sequence Listing as discussed below. The remaining amendments to the specification correct typographical and grammatical errors. No new matter is introduced with any of the foregoing amendments.

In amendments, claims 1-25, 35-41 and 56-74 have been canceled. Claims 27, 30, 32 and 33 were previously canceled. As suggested by the Examiner, the claims are amended to specify sequence information by reference to the Sequence Listing. Support for the use of the term "glial cell line-derived neurotrophic factor" can be found on page 33, line 18 and Example 1, page 43.

New Claims 75-88 have been added. Support for Claims 75 and 87 may be found on page 20, lines 9-27. Specific support for Claims 80, 81, 84 and 85 may be found on page 42, line 9 through page 43, line 11. Support for Claim 83 may be found on page 42, lines 9-17. Support for Claim 88 may be found on page 19, lines 33-35. Support for the remaining newly added claims may be found throughout the specification.

## Informalities

The disclosure is objected to on the basis that the sequences disclosed in the Figures should be referenced by SEQ ID NO in the Brief Description of the drawings. By the foregoing amendments, the Brief Description of the Drawings has been amended to refer to the appropriate SEQ ID NOS just as they are used throughout the remainder of the specification.

The Examiner also expressed the belief that the precursor protein amino acid sequences do not appear in the present Sequence Listing. The Applicants respectfully submit that the pre-pro sequences are contained in the Sequence Listing. The Examiner's attention is directed to page 67, lines 12-23, wherein the nucleic acid sequence and the encoded amino acid residues 1-50 of human pre-pro GDNF are discussed and illustrated by Figure 22 and SEQ ID NO:8. The last 26 codons of the pre-pro segment are depicted in Figure 19 and SEQ ID NO:5. The combination of these pre-pro sequences clearly provides the full pre-pro sequence of GDNF. Also as discussed on page 67, lines 5-23, codon 51 is split by an intron. The combination of Figures 22 and 19 illustrates that the codon is TCA encoding Ser. Thus, the pre-pro segment of 77 codons is fully illustrated in the specification, both by nucleic acid and amino acid sequences.

To further clarify the description of the present invention, an additional SEQ ID NO (i.e., SEQ ID NO:25) may be included in the specification. SEQ ID NO:25 presents the pre-pro sequences found in SEQ ID NOS:8 and 5 in combined form to illustrate the nucleotide and amino acid sequences for human pre-pro GDNF in a single Sequence Listing (exclusive of the intron that splits codon 51). The new Sequence Listing does not contain any new matter because the nucleotide and amino acid sequences of SEQ ID NO:25 are fully supported by the combination of original Figures 22 and 19 as well as SEQ ID NOS:8 and 5 as discussed above. To further mitigate any confusion regarding human pre-pro GDNF, SEQ ID NO:5 may be amended in accordance with 37 CFR 1.822(d) to insert the amino acid (Ser) encoded by codon 51 (TCA). A printed version of a revised Sequence Listing is attached to this response and may be inserted into the Specification as pages 92-99D. Also submitted herewith is a new diskette and a new Statement under 37 CFR 1.821(f) containing the revisions discussed above.

## Section 112, First Paragraph Rejections

Claims 26, 28-29, 31, 34-36 and 42-55 were rejected under §112, first

paragraph, as non-enabled. The basis for the rejection is that the disclosure is allegedly enabling only for claims limited to nucleic acid sequences encoding the glial cell line-derived neurotrophic factor (GDNF) amino acid sequences of Figures 13 and 19, i.e., SEQ ID NOS: 3 and 5. The Examiner bases this rejection on the statement that the definition of GDNF-encoding nucleic acid sequences in the specification is phrased only in terms of biological equivalents and that the specification does not adequately describe how to identify any other nucleic acid sequences which encode GDNF.

Applicants respectfully traverse this rejection. Applicants direct the Examiner's attention to the following portions of the specification which include specific descriptions of a variety of nucleic acids that encode GDNF:

(1) nucleic acid sequences that encode the rat or human mature (as well as pre-pro) GDNF proteins set forth in SEQ ID NOS: 3, 4, 5 and 8 (please see page 29, line 33 through page 30, line 10);

(2) nucleic acid sequences that encode a polypeptide that is at least 70% homologous to amino acid sequences of SEQ ID NOS:4 and 6 (please see page 20, lines 5 through 27);

(3) nucleic acid sequences that hybridize to the nucleotide sequences set forth in SEQ ID NOS: 3, 4, 5 or 8, or their complements (please see page 30, lines 11 through 24; page 34, line 26 through page 36, line 22);

(4) nucleic acid sequences that encode a polypeptide that is identified by means of an anti-GDNF antibody (please see page 30, lines 24 through 27); and

(5) nucleic acid sequences identified by polymerase chain reaction techniques (please see page 36, line 23 through page 37, line 26).

As demonstrated by these teachings throughout the specification, the definition of GDNF-encoding nucleic acid sequences is not phrased merely in terms of biological equivalents.

Each of the claimed sequences is clearly enabled by the Applicants' discovery and description of the nucleic acid and amino acid sequences of mature or full length GDNF as well as the pre-pro form of GDNF. Moreover, the description of the nucleic acid sequence identification methods, as well as the description of how to determine whether the expressed polypeptide increases dopamine uptake by dopaminergic neurons, are readily used by one of ordinary skill in the art to duplicate the claimed invention without undue experimentation. Thus, the specifically claimed nucleic acid sequences are enabled by the specification.

In contrast to the case of *Ex parte Maizel* [27 USPQ2d 1662, 1665 (1993)] cited by the Examiner, the claimed nucleic acid sequences are supported by the present specification and are not solely defined by the biological function of the encoded protein. To further clarify the correlation between the supporting disclosure and the claims, the claims

have been amended to include the pertinent descriptions of the nucleic acid sequences. The claim language is directly supported by the specification, as discussed above, and introduces no new subject matter. The scope of the claims, therefore, is commensurate with the scope of the enabling disclosure, and Applicants have provided a clear basis for the suitability or operativeness of the various identification and testing means included within the specification. Because Applicants have demonstrated the reasonable correlation between the description and the claims, Applicants respectfully submit that this §112, first paragraph rejection may properly be withdrawn.

The Examiner raised the question of whether SEQ ID NO:8 encodes an active protein. As described above and in the specification, SEQ ID NO:8 encodes the first 50 amino acids of the pre-pro segment of human GDNF. Those of ordinary skill in the art will appreciate that this partial pre-pro sequence itself is not expected to have GDNF activity. The nucleic acid sequence of SEQ ID NO:8 may be useful, however, in identifying other members of the GDNF family, as provided at page 34, line 21 to page 37, line 26. Also, of course, SEQ ID NO. 8 would be useful in constructing a human pre-pro GDNF or a nucleic acid encoding same.

With respect to claim 36, the Examiner objected to the use of the term "dopaminergic activity". Claim 36 has been canceled in view of the amendments made to the remaining claims. The term is not used in the remaining claims, and therefore, the objection may properly be withdrawn.

With respect to claim 44, the Examiner objected to the phrase "conditions for amplification of the vector". The claim amendments have also removed this term, and again the objection may properly be withdrawn.

Section 112, Second Paragraph Rejections

Claims 28, 31 and 35-36 were rejected under §112, second paragraph, as indefinite. Claims 35 and 36 have been canceled. Claim 28 is alleged to be indefinite because the Examiner believes it is not clear whether the claim is directed to the nucleic acid sequence encoding only the human mature or full length GDNF protein, or the pre-pro form of the protein. It is believed that the foregoing amendment to claim 28, as suggested by the Examiner, adequately addresses this rejection. Support for this amendment can be found in the specification at page 13, lines 20-23 and SEQ ID NO: 5.

Claim 31 is allegedly unclear because the Figures and SEQ ID NOS. to which it refers contain both DNA and amino acid sequences, and because Figure 22 and SEQ ID NO:8 provide only a portion of the pre-pro GDNF sequence. It is believed that the foregoing amendments and discussion also address these issues. Support for the amendment to pending claim 31 can be found in Figures 19 and 22 and original SEQ ID NOS:5 and 8.

Claim 31 is alleged to be confusing in that it appears to claim two DNA sequences simultaneously rather than in the alternative. This situation has been remedied by reference to SEQ ID NO:25 rather than the combination of SEQ ID NOS:5 and 8. As discussed above, SEQ ID NO:25 sets forth the nucleic acid sequence of pre-pro human GDNF without the intron in codon 51 of the pre-pro segment, as well as the amino acid sequence. No new matter is introduced with this additional sequence as the content was already in the specification and in SEQ ID NOS:5 and 8.

With the above-referenced amendments, the claims have been clarified to refer to specific nucleic acid sequences which encode mature or full length GDNF protein. It is therefore respectfully requested that these §112, second paragraph, rejections be withdrawn.

Section 102 Rejection

Claims 26, 29, 34, 42-43 and 50-54 were rejected under section 102(b) over Monard et al. (EP 233 838) which the Examiner alleges describes the nucleic acid sequences of the present invention. Applicants respectfully traverse this rejection.

The Examiner's basis for rejection is that Monard et al. discloses human and rat glial-derived neurite-promoting factor (GdNPF) in both precursor and mature forms, and DNA sequences encoding same. Monard et al. also reportedly teach recombinant expression of the GdNPF protein in *E. coli* and COS-7 cells. The Examiner reasons that the present specification describes a "glial cell line-derived neurotrophic factor" (GDNF) as encompassing any glial cell-produced protein with neurotrophic activity, and that the GdNPF-encoding nucleic acids of Monard et al. falls within the scope of the rejected claims.

Applicants respectfully traverse this rejection. As discussed above, the claims have been amended to describe specific nucleic acid sequences. Applicants have not detected, nor has the Examiner pointed out any correspondence between the presently claimed GDNF-encoding nucleic acid sequences and the GdNPF-encoding nucleic acid sequences of Monard et al. This lack of correspondence is clear evidence that Monard et al. failed to describe the nucleic acid sequences of the present invention. Thus, Monard et al. is not a valid §102(b)

reference as it does not teach, i.e., identically describe, each and every element of the rejected claims. In view of the foregoing, it is respectfully submitted that the Monard et al. reference fails to anticipate the pending claims, and therefore, if is requested that this rejection be withdrawn.

The Section 103 Rejections

Claim 44 stands rejected under §103 as obvious over Monard et al. in view of Wurm et al., *Biologicals*, 18:159-164 (1990). The Examiner's basis for the rejection is that claim 44, which is directed to a method of expression and amplification of a nucleic acid encoding GDNF, is obvious because Wurm et al. teaches an amplification method.

Wurm et al. discusses the amplification of transfected genes in Chinese Hamster Ovary cells. Applicants respectfully point out that Wurm et al. do not render claim 44 obvious because claim 44 recites a recombinant DNA method for production of GDNF, and neither Wurm et al. nor Monard et al. teach or suggest the claimed GDNF nucleic acid sequence. As discussed above, the GdNPF nucleic acid sequence of Monard et al. is not the GDNF nucleic acid sequence, nor is GdNPF known to have the ability to induce dopamine uptake by substantia nigra dopaminergic neurons or the ability to promote survival of parasympathetic or sympathetic nerve cells. Wurm et al. make no reference at all to a glial cell line-derived neurotrophic factor, nor does it provide any information to correct the deficiencies of Monard et al. Therefore, it is respectfully submitted that *prima facie* obviousness of claim 44 has not been established and that the rejection should properly be withdrawn.

Claim 55 stands rejected under §103 over Monard et al. and Olson et al. (U.S. 4,518,526). Claim 55 is directed to refolding GDNF expressed in *E. coli*. The Examiner states that Olson et al. teach the solubilization of refractile bodies and the refolding the recombinant proteins contained therein. Applicants respectfully traverse this rejection.

Olson et al. describe a method for enhancing protein deposition in refractile bodies, isolating refractile bodies, solubilizing the protein in the refractile bodies, purifying the protein, refolding the protein and oxidizing the protein to properly reform di-sulfide bonds. Olson et al., however, do not refer to or suggest a glial cell line-derived neurotrophic factor or the presently claimed nucleic acid sequences encoding GDNF. Therefore, Olson et al. fail to provide the teachings that are missing from Monard et al. concerning the GDNF nucleic

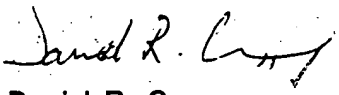
acid sequences. In view of the failure of Monard et al. and/or Olson et al. to teach or suggest the claimed GDNF nucleic acid sequences, it is submitted that *prima facie* obviousness has not been established and that this rejection should also properly be withdrawn.

## Allowable Subject Matter

Applicants acknowledge with thanks the Examiner's indication that claims 28, 31 and 35-36 were allowable over the prior art of record. As discussed above, these claims have been amended or incorporated into the remaining claims to address the §112, second paragraph rejections raised by the Examiner and contain the amendments suggested by the Examiner.

For the foregoing reasons and in view of the amendments, Applicants submit that this application is in condition for allowance, and such favorable reconsideration is earnestly requested. If the Examiner has further questions concerning the application, the Applicants' representative would appreciate the opportunity to talk with the Examiner, on the telephone or in person, to facilitate the prosecution of the application.

Respectfully submitted,



Daniel R. Curry
Attorney for Applicants
Registration No: 32,727
Phone: (805) 447-8102
Date: January 5, 1996

Please send all future correspondence to:

U.S. Patent Operations/DRC
M/S 10-1-B
AMGEN INC.
Amgen Center
1840 Dehavilland Drive
Thousand Oaks, California 91320-1789